PREPARATIVE AND INDUTRIAL SCALE ISOLATION AND PURIFICATION OF POLYUNSATURATED FATTY ACIDS (OMEGA-3 AND OMEGA-6)

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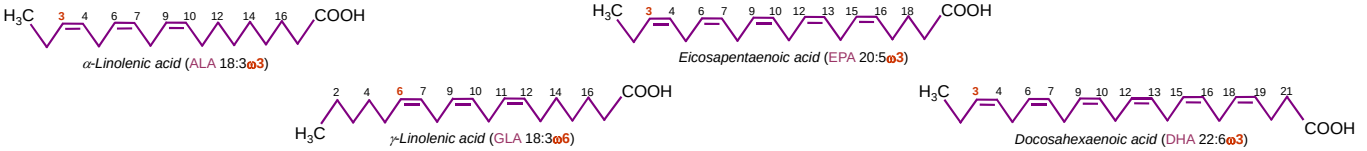
INTRODUCTION

Long-chain omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) have become an important subject in both the scientific community and our everyday life, and we encounter them in pharmaceutical and/or health applications as well as in food applications. Among these PUFA's have attracted especial attention, due to their role in human health and nutrition. Essential fatty acids cannot be synthesized *de novo* by humans and therefore, need to be obtained from the diet. With the growing public awareness of the nutritional benefits of PUFA's, the market for such products is expected to grow in the future. Docosahexaenoic (DHA), eicosapentaenoic (EPA) and gamma linoleic (GLA) acids are the mostly used PUFA in nutraceuticals and functional foods. They are being used in wide array of products ranging from dietary supplements to infant formulas.

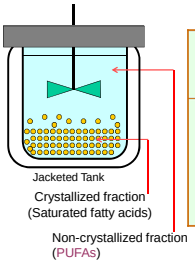
Naturally these fatty acids are associated with other lipophilic compounds and effective separation and isolation techniques are needed to recover them in concentrated forms. With the growing public awareness of the nutritional benefits of PUFAs, the market for such products is expected to grow in the future. This presentation focuses on some of the new methodologies that we'll be able to provide in addition to the capabilities at present.

Omega-3 (ω3) and Omega-6 (ω6) Fatty Acids



Low Temperature Crystallization

- Utilizes the melting point (MP) characteristic of fatty acids, which depends on chain length and degree of unsaturation.
- As the chain length increases, MP of fatty acids increases, however, for longer chain fatty acids, unsaturation, results in a decrease of MP.
- At low temperature, short chain fatty acids crystallize and PUFAs can be isolated from the rest of the fatty acids.
- Organic solvents (*eg.* Hexanes, Acetone) facilitate separation of fatty acids during crystallization.



Enrichment of ω3 Fatty Acids by Low Temperature Crystallization

Fatty Acid Esters	Starting Marine Oil	Hexanes		Acetone	
		TAG	FFA	TAG	FFA
EPA (%)	6.8	8.2	11.8	12.0	13.8
DHA (%)	8.4	10.1	12.5	15.1	17.5
Total ω3 (%)	22.2	27.8	31.6	37.2	40.6

Conditions

- Marine oil (TAG or FFA)
- Solvents: Hexanes or Acetone
- Oil-to-solvent ratio: 1:4 (w/v)
- Temperature: -25ºC
- Time: 24h with slow mixing
- Separate crystals by filtration
- Evaporate solvent from liquid fraction (non-crystallized fraction)

Fractional Distillation

- Fractional distillation uses the differences in the boiling point and molecular weight of fatty acid esters under vacuum (0.1-4.0 mmHg) and high temperatures (150-210ºC).
- Table shows the conditions and results of fractional distilled marine oil ethyl esters (EE).
- By fractional distillation, more than doubling of the Omega-3 fatty acids in the isolate (non-distilled fraction) can be achieved.

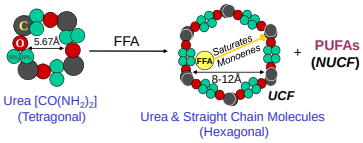
Enrichment of ω3 Fatty Acid-Ethyl Esters by Fractional Distillation

Fatty Acid Esters (%)	Starting Marine Oil Esters	Trial-1	Trial-2
EPA-EE	7.0	11.8	11.8
DPA-EE	4.2	7.0	12.2
DHA-EE	8.8	15.5	26.2
Total ω3-EE	23.2	38.2	53.1
Yield (%)		50	23

Conditions	Trial-1	Trial-2
Temperature (ºC)	170	190
Vacuum (mm Hg)	1.5	1.5
Holding Time (min)	7	7

Urea Complexation

- In a free fatty acid (FFA) mixture, saturated and monounsaturated fatty acids can be complexed with urea.
- Presence of double bonds increases the bulk of the molecule and reduces the likelihood of its complexation with urea, *i.e.* PUFAs remain in the liquid and are referred to as non-urea complexing fraction (*NUCF*).



Enrichment of ω3 and ω6 Fatty Acids by Urea Method

Values are in %	Algal	Borage	Flax	Marine		
	DHA	GLA	ALA	EPA	DPA	DHA
Starting oil	57.1	21.8	57.2	6.8	4.2	8.4
After urea	98.8	88.1	80.1	10.9	2.4	67.6
Urea-to-FFA Ratio (w/w)	4:1	3:1	2:1		3:1	

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